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Significant progress has been made in analyzing the anatomical makeup of the human suprachiasmatic nucleus (SCN) and adjacent structures. The largest population of neurons in the human SCN are neuropeptides containing neurons. The human SCN also contains vasopressin, vasoactive intestinal polypeptide and neuropeptide Y. Development of DiI methods to study connections between neuronal populations has been disappointing and new approaches are now being explored.		14. SUBJECT TERMS	
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**AIR FORCE OFFICE OF SCIENTIFIC RESEARCH  
ANNUAL TECHNICAL REPORT**

**ORGANIZATION OF THE HUMAN CIRCADIAN SYSTEM**

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## TECHNICAL REPORT

**Objectives:** The overall objective of this project is a detailed description of the organization of the primate circadian timing system. The specific objectives for this project year were as follows: 1) Immunohistochemical analysis of the human SCN; 2) Immunohistochemical analysis of the human LGN; 3) Analysis of retinal projections to the hypothalamus and lateral geniculate in the macaque monkey; 4) Development of DiI methodology to study connections in postmortem human material.

**Status of Research Effort:** This will be described for each of the objectives noted above. The material analyzed during this year included 8 human hypothalami and 4 lateral geniculates prepared for immunohistochemistry, 6 human hypothalami and 2 lateral geniculates used for DiI studies and one macaque monkey used for CT-HRP analysis of retinal projections and immunohistochemical analysis. It was anticipated that 2 monkeys would be used but recently imposed restrictions on importation raised animal prices so much that only one was feasible.

**Human SCN- Immunohistochemical Analysis.** The human hypothalami prepared and studied this year included tissue from males and females and from young adult to aged individuals. All were obtained from routine postmortem specimens fixed in buffered formaldehyde. Sections were cut in the coronal plane and a control series was stained routinely with cresyl violet for cytoarchitectonic analysis. Series of sections were stained with a variety of antisera including ones to vasopressin (VP), neuropeptide Y (NPY), neurotensin (NT), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), luteinizing hormone releasing hormone (LHRH), galanin (GAL) and the calcitonin gene-

related peptide (CGRP). The human SCN is best described by a characterization of neurons containing VP, NT, VIP and NPY. The largest population of neurons in the SCN is those containing NT. They extend from the rostral to the caudal borders of the nucleus and are present throughout its extent. The SCN is the first distinct nucleus to appear in the human hypothalamus. NT neurons are present in the optic chiasm and adjacent lamina terminalis at the very rostral portion of the 3rd ventricle. At this level there is no evident nucleus in Nissl material. More caudally the NT neurons are present in an extended ovoid area along the wall of the 3rd ventricle and running from the paraventricular nucleus dorsally to the supraoptic nucleus laterally. At caudal levels, the SCN loses continuity with the optic chiasm and gradually fuses into the adjacent anterior hypothalamic area and retrochiasmatic area. The second largest population of neurons is the one containing VP. Like the NT neurons these have a very wide distribution through the SCN. There is one difference between the two populations with respect to distribution; VP neurons are not present in the ventral region of the SCN adjacent to the optic chiasm, the presumptive area of retinohypothalamic tract termination. VP neurons appear to be a population separate from the NT neurons. NPY neurons are present in the center of the SCN, overlapping the area free of VP neurons. They are associated with a very fine plexus of axons, distinct from the coarse axons in the surrounding hypothalamus. The smallest population of identified neurons in the SCN is those containing VIP. These are entirely confined to the ventral region of the nucleus but there are axons extending dorsally and laterally into the adjacent anterior hypothalamus. VIP has presented the greatest difficulty in obtaining good material and we require more material to have definitive data on this population.

Two other observations are worth noting. First, the SCN appears quite variable in its location largely as a function of the anatomy of the optic chiasm. Variations in chiasmal anatomy

are well known and this is not surprising. Second, there is considerable variation among individuals in the number of any of the individual peptide - containing populations and this needs to be quantified and described in detail. On initial observation, it does not appear to be a function of sex or age.

Immunohistochemical analysis of the human LGN. The material prepared thus far has been done with cresyl violet and antisera against VIP, NPY, substance P (SP) and enkephalin (ENK). The LGN has two clear cytoarchitectonic subdivisions in the human, the dorsal lateral geniculate nucleus (DLG) and the perigeniculate nucleus (PG). The DLG has no structures stained with any of the antisera used. The PG has two distinct subdivisions, a dorsal and lateral cell group that is continuous with the ventral part of the thalamic reticular nucleus and a medial and ventral cell group continuous medially with the zona incerta. The dorsal and lateral cells also have no immunohistochemically identified structures. The medial and ventral area contains a number of NPY and ENK immunoreactive perikarya and axons. Those are distributed fairly evenly over the entire nucleus. It is clear that more information is required to identify which component is homologous to intergeniculate leaflet and which is homologous to the ventral lateral geniculate of non-primate mammals. It is expected that the monkey data will be very useful in this regard.

Retinal projections in the macaque monkey. Cholera toxin conjugated to ARP (CT-HRP) was injected into the vitreous of one eye in a adult macaque monkey and the pattern of transport studied histochemically. Retinohypothalamic projections are to present to the SCN, adjacent anterior hypothalamus, lateral hypothalamus and retrochiasmatic area. The projection to SCN is dense in the ventral region of the nucleus and substantially greater on the side ipsilateral to the eye

injection. There is a minor extension of the projection into the adjacent anterior hypothalamic area and a small, but clear, terminal field in the lateral hypothalamic area, both substantially greater on the ipsilateral than the contralateral side. At the caudal end of the SCN, the terminal fields expand on both sides and there is a fairly extensive field in the retrochiasmatic area. In general, this pattern of retinal projections appear quite similar to the rat, and very different from the hamster. This material is very recently obtained, surgery and perfusion in November, and the analysis of geniculate projections has not been completed. However, a preliminary analysis indicates that this will serve in large measure to facilitate definition of the IGL in the monkey. In addition, sections from the monkey material have been prepared using many of the antisera noted above. This has just been completed and the material has not yet been studied.

Development of DiI methodology: Thus far, this has been a disappointing project. In specimens in which DiI was placed in the optic nerves-chiasm adjacent to the hypothalamus, or in the primary optic tract adjacent to the lateral geniculate, only very limited transport of DiI has been observed. This is in accord with observations of other investigators who have not found significant transport of the dye in adult material, particularly in myelinated pathways. For this reason, and because of the distances involved for transport, it seems unlikely this method will work for the lateral geniculate. In contrast, it does appear reasonable to conclude that it would work in the hypothalamus where retinal projections are probably unmyelinated. The methodology has been modified to improve transport by keeping the specimens in an oven at 37°C. It seems likely that something in the range of 4-6 months will be required for transport and several specimens are in process at this time.

Publications: No publications have appeared as yet. One publication, entitled "Organization of the Human Circadian System," will be published in *Progress in Brain Research*. This is in preparation and will be taken from a presentation to be given at a symposium on "The Human Hypothalamus in Health and Disease" in Amsterdam, August, 1991.

Professional Personnel: Robert Y. Moore, M.D., Ph.D.

Papers Presented: Moore, R.Y. and Speh, J.C. "The Human Suprachiasmatic Nucleus." Society for Neuroscience Abstracts 16:602, 1990

Inventions, Patents: None